MOLECULAR WEIGHT OF β-LACTOGLOBULIN AS DETERMINED BY LIGHT-SCATTERING MEASUREMENTS

The light-scattering method of Debye (1) has been used to determine the molecular weight of β-lactoglobulin. An absolute turbidimeter (2) was used. The lactoglobulin was prepared from milk by the method of Sorensen and Sorensen (3). It was recrystallized immediately before use. Isoelectric solutions in 0.1 *M* phosphate buffer of pH 5.2 were filtered by pressure through an ultrafine sintered-glass filter. Turbidities were determined for a series of protein concentrations, at wavelengths 436 and 546 mμ. The turbidity of the buffer was subtracted from that of each solution. Protein concentrations were determined by drying aliquots to constant weight and subtracting the corresponding weight of buffer solids. The function Hc/τ was plotted against c (Fig. 1). $H = 32\pi^3 n_o^2 [(n-n_o)/c]^2/$

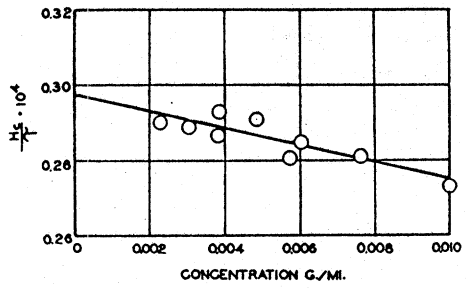

FIG. 1. Light scattering of lactoglobulin solutions as a function of concentration. Each point is an average of one determination at 436 mμ and one at 546 mμ.

$3N\lambda^4$; c = protein concentration, g./ml.; τ = turbidity, cm.$^{-1}$; n_o = refractive index of buffer; n = refractive index of protein solution; λ = wavelength of light, cm.; N = Avogadro's number. The specific refractive increment, $(n-n_o)/c$, was determined with a differential refractometer (4). The values obtained at 25°C. were 0.189 at 436 mμ and 0.183 at 546 mμ. These were obtained in 0.1 *M* NaCl at pH 5.2, but there is no reason to expect that the values in 0.1 *M* phosphate buffer of the same pH would be significantly different. The values agree well with those of 0.1892 and 0.1818, respectively, found by Pedersen (5) in 0.5 *M* NaCl. Values of 0.193 and 0.186 were calculated from data of Perlmann and Longsworth (6). The cause of the difference between these and the values used in this

paper is not clear at present, but probably lies either in the method of determining protein concentration or in the purity of the sample.

Values of Hc/τ at the two wavelengths differed by about 8%, the 546 mμ values being higher. Average values are plotted in Fig. 1. Extrapolation to zero concentration of the best straight line through these points, determined by the method of least squares, gives the reciprocal of the molecular weight, which, thus determined, was 33,700. This figure has been corrected for the depolarization of the scattered light, which was found to be $\rho_v = 0.015$.

Bull and Currie (7) have summarized previous determinations of molecular weight of β-lactoglobulin. The values obtained most frequently were roughly 42,000 and 35,000. The present value lends weight to the 35,000 group. Recently Senti and Warner (8) have redetermined the molecular weight by the X-ray diffraction method, obtaining 35,400 on wet crystals and 35,600 on air-dried crystals.

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